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Applicant:	Sontheimer, *et al.*	Examiner:	Chen, Shin-Lin
Serial No.:	10/686,782	Art Unit:	1632
Filing Date:	October 17, 2003		
Title:	Diagnosis and Treatment of Neuroectodermal Tumors		

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

DECLARATION UNDER 37 C.F.R. § 1.132

I, Alison M. O'Neill, M.D., declare as follows:

1. I am the Vice President, Medical Affairs at TransMolecular, Inc., Cambridge, Massachusetts, the assignee of United States patent application Serial No. 10/686,782, filed October 17, 2003, and entitled "Diagnosis and Treatment of Neuroectodermal Tumors". A copy of my curriculum vitae is attached hereto as **Exhibit A.**

2. I have reviewed the specification and claims of the above-referenced patent application as well as the Office Action mailed November 21, 2006, and understand that the Examiner has rejected all the pending claims for failing to comply with the enablement requirement. I further understand that the Examiner has questioned whether a chlorotoxin fused to a cytotoxic moiety (1) can pass through the blood-brain barrier in a subject, and (2) can be administered to provide therapeutic effect *in vivo*.

3. One purpose of the present Declaration is to confirm assertions made in the Specification that chlorotoxin fused to a cytotoxic moiety passes through the blood-brain barrier when administered to a subject, and has a therapeutic effect *in vivo*. Another purpose of this

Declaration is to confirm that binding of a chlorotoxin-cytotoxic moiety complex to neuroectodermal tissue correlates with therapeutic activity.

4. A synthetic version of chlorotoxin (TM-601) has been manufactured and covalently linked to iodine 131 (^{131}I-TM-601), a cytotoxic moiety. Preclinical studies and Phase I clinical trials have been completed, under my supervision, in patients with recurrent high-grade glioma. These studies demonstrated that intracavitary dosing of ^{131}I-TM-601 appears safe, minimally toxic, and binds malignant glioma with high affinity and for long durations. Some of the results obtained in this study have been reporting in A.N. Mamelak *et al.*, "Phase I Single-Dose Study of Intracavitary-Administered Iodine-131-TM-601 in Adults With Recurrent High-Grade Glioma", Journal of Clinical Oncology, August 1, 2006, 24: 3644-3650 ("Mamelak") (see **Exhibit B**). As of February 2007, out of the 18 patients that have received a single dose of ^{131}I-TM-601 in the Phase I trial, 5 survived 12 months or longer from recurrence; 2 survived more than 36 months from recurrence; and 1 patient remains alive (more than 4 years from recurrence). These results confirm the assertion made in the Specification that chlorotoxin with radioactive moieties selectively bind to gliomas and expose cells to high levels of radioactivity and can therefore be used to treat gliomas (see last sentences of Example 21 of the parent U.S. Pat. No. 5,905,027, which is incorporated by reference in the instant Application).

5. A Phase II trial of ^{131}I-TM-601 using higher doses of radioactivity and repeated intracavitary administrations to patients with high-grade glioma is underway, under my supervision. In the Dose Escalation Phase of this trial, patients received ^{131}I-TM-601 at 0.4mg/20mCi, repeated 3 times at 7 day intervals, ^{131}I-TM-601 at 0.6mg/30mCi, repeated 3 times at 7 day intervals, ^{131}I-TM-601 at 0.8mg/40mCi, repeated 3 times at 7 day intervals or ^{131}I-TM-601 at 0.8mg/40mCi, repeated 6 times at 7 day intervals. In the Randomized Phase of this trial, patients have received or are receiving ^{131}I-TM-601 at 0.8mg/40mCi, repeated 3 times or 6 times at 7 day intervals. **Exhibit C** is a graph showing the length of survival (determined in April 2007) for each patient enrolled in this Phase II trial, after treatment with ^{131} I-TM-601. As of April 2007, of the patients who had been on study for 6 months, 86% (31 out of 36 patients) remained alive, of the patients who had been on study for 9 months, 63% (17 out of 27 patients) remained alive, and of the patients who had been on study for 12-months, 45% (10 out of 22

patients) remained alive. These results demonstrate the therapeutic effect of a chlorotoxin-cytotoxic moiety complex *in vivo*.

6. In addition, enrollment has begun in a Phase I trial evaluating the biodistribution and safety of systemic delivery of ^{131}I-TM-601 to patients with recurrent or refractory primary solid tumors (including malignant glioma) with metastatic involvement (including brain metastases). In this trial, patients receive an intravenous dose of ^{131}I-TM-601 at 0.2mg/10mCi, and, if necessary, a second intravenous dose of ^{131}I-TM-601 at 0.4mg/20mCi, to allow for tumor localization. Patients whose tumor is localized then receive an intravenous treatment dose of ^{131}I-TM-601 at 0.6mg/30mCi. Preliminary results showed that intravenous administration of ^{131}I-TM-601 resulted in tumor-specific localization of ^{131}I-TM-601 in 5 out of 5 patients with malignant glioma, 1 out of 1 patient with prostate cancer, 1 out of 1 patient with Non-Small Cell Lung cancer, 1 out of 2 patients with metastatic melanoma, and 1 out of 1 patient with colon cancer. These results confirm that a chlorotoxin-cytotoxic moiety complex can reach the target site when administered to a patient, and further demonstrate that such a complex can pass through the blood-brain barrier to reach a tumor localized in the brain.

7. Furthermore, one patient with malignant glioma, who showed tumor-specific uptake of ^{131}I-TM-601 and who then received the intravenous treatment dose, was found to exhibit evidence of radiographic improvement (see **Exhibit D**, which shows a set of Magnetic Resonance (MRI) images recorded from that patient before and after ^{131}I-TM-601 treatment). Another patient with malignant glioma, who also showed tumor-specific uptake of ^{131}I-TM-601 and who then received the intravenous treatment dose, exhibited apparent clinical improvement in the absence of imaging improvement. These results demonstrate the therapeutic effect of a chlorotoxin-cytotoxic moiety complex *in vivo*.

8. The Specification teaches that binding of a chlorotoxin-cytotoxic moiety complex to neuroectodermal tumor tissues correlates with therapeutic activity *in vivo*. All the clinical trial data presented in the present Declaration confirm this correlation.

9. I, Alison M. O'Neill, declare that all statements made herein of my own knowledge are true and that these statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful, false statements and the like are made punishable by fine or imprisonment, or both, under §1001 of Title 18 of the United States Code and that such willful, false statements may jeopardize the validity of the application or any patents that may issue thereon.

Alison M. O'Neill, M.D.

May 21, 2007

Date

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EDUCATION

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1994 - 1996	Fellowship, Neuro-Oncology Memorial Sloan-Kettering Cancer Center New York, New York
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2002 - present	Licensed physician, Commonwealth of Massachusetts
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PROFESSIONAL CERTIFICATIONS

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Exhibit A

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HOSPITAL APPOINTMENTS

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1996 – 2002	Active Staff Physician, Neurology Service University of Alabama Hospitals
1996 – 2002	Consultant and Attending Neurologist, Birmingham VA Medical Center
1996 – 2002	Staff Physician Cooper Green Hospital, Birmingham, AL

PROFESSIONAL SOCIETIES

1997 – present	Society for Neuro-Oncology
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HONORS AND AWARDS

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1990	Alpha Omega Alpha Honor Medical Society University of Chicago Pritzker School of Medicine
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JOURNAL OF CLINICAL ONCOLOGY | ORIGINAL REPORT

Phase I Single-Dose Study of Intracavitary-Administered Iodine-131-TM-601 in Adults With Recurrent High-Grade Glioma

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Authors' disclosures of potential conflicts of interest and author contributions are found at the end of this article.

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ABSTRACT

Purpose
TM-601 binds to malignant brain tumor cells with high affinity and does not seem to bind to normal brain tissue. Preclinical studies suggest that iodine-131 (^{131}I) –TM-601 may be an effective targeted therapy for the treatment of glioma. We evaluated the safety, biodistribution, and dosimetry of intracavitary-administered ^{131}I-TM-601 in patients with recurrent glioma.

Patients and Methods
Eighteen adult patients (17 with glioblastoma multiforme and one with anaplastic astrocytoma) with histologically documented recurrent glioma and a Karnofsky performance status of ≥ 60% who were eligible for cytoreductive craniotomy were enrolled. An intracavitary catheter with subcutaneous reservoir was placed in the tumor cavity during surgery. Two weeks after surgery, patients received a single dose of ^{131}I-TM-601 from one of three dosing panels (0.25, 0.50, or 1.0 mg of TM-601), each labeled with 10 mCi of ^{131}I.

Results
Intracavitary administration was well tolerated, with no dose-limiting toxicities observed. ^{131}I-TM-601 bound to the tumor periphery and demonstrated long-term retention at the tumor with minimal uptake in any other organ system. Nonbound peptide was eliminated from the body within 24 to 48 hours. Only minor adverse events were reported during the 22 days after administration. At day 180, four patients had radiographic stable disease, and one had a partial response. Two of these patients further improved and were without evidence of disease for more than 30 months.

Conclusion
A single dose of 10 mCi ^{131}I-TM-601 was well tolerated for 0.25 to 1.0 mg TM-601 and may have an antitumoral effect. Dosimetry and biodistribution from this first trial suggest that phase II studies of ^{131}I-TM-601 are indicated.

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INTRODUCTION

Despite aggressive efforts, the prognosis for survival from malignant glioma has not significantly improved in the last 20 years.[1-5] The 5-year survival for glioblastoma remains approximately 3%, and the 2-year survival is approximately 8.2%.[2] TM-601 is a synthetic version of a peptide (chlorotoxin) found in the venom of the giant yellow Israeli scorpion *Leiurus quinquestriatus*.[6] This 36–amino acid peptide has been explored[7,8] as a candidate for targeting gliomas. TM-601 crosses blood-brain and tissue barriers[7] and binds to a phosphatidyl inositide, a phosphorylated lipid on lamellipodia of tumor cells.[9] Preclinical studies demonstrated the stability, safety, efficacy, and lack of immunogenicity of radioiodinated TM-601. We performed a phase I study to evaluate the safety, biodistribution, and dosimetry of intracavitary iodine-131 (^{131}I) –TM-601 in adult patients with recurrent high-grade glioma.

PATIENTS AND METHODS

Preparation of ^{131}I-TM-601

TM-601 (lyophilized, sterile, and pyrogen free) was radiolabeled with 10 mCi ^{131}I via the iodogen bead method[10] at the clinical site and used within 78 hours (typically < 2 hours). Release specifications required less than 5% free iodine (by instant thin-layer chromatography) and no pyrogenicity.

Exhibit B

Patients and Treatment Protocol

This study was performed in accordance with the Declaration of Helsinki and with the approval of the US Food and Drug Administration and of the institutional review boards at each participating site. Informed consent was obtained from each patient before participation.

Adult (> 18 years) patients with histologically documented supratentorial malignant glioma, a Karnofsky performance status (KPS) of ≥ 60%, and a life expectancy of at least 3 months were eligible for this trial.[11] Patients were required to have unifocal tumors that progressed at the site of original disease after standard of care. Additional inclusion criteria included tumor cross-sectional diameter of less than 6 cm; no direct communication with the ventricle; no previous immunotherapy, gene therapy, implantable chemotherapy, or stereotactic radiosurgery; and at least 6 weeks from the last dose of nitrosourea-containing chemotherapy. Eligible patients underwent tumor debulking surgery; pathologic confirmation of high-grade glioma was performed for all patients. During surgery, a ventricular access device (Rickham or Ommaya reservoir) was placed in the tumor cavity. Patients recovered from surgery for 14 to 28 days before undergoing treatment with the study drug. This waiting period was chosen to avoid confounding between neurologic deficits after surgery and adverse events attributed to the study drug. Patients were excluded from the trial if it was deemed by the investigator that they suffered a surgical complication that made proceeding with treatment unsafe or if the KPS was less than 60%.

Magnetic Resonance Imaging

Details of all imaging methods are reported elsewhere.[12] Briefly, postoperative magnetic resonance imaging (MRI) scans were acquired for each patient before ^{131}I-TM-601 injection. Sequences included 3-mm thick coronal, axial, and sagittal T1-weighted images (with and without gadolinium contrast), axial and coronal T2, axial fluid attenuated inversion recovery (FLAIR), and three-dimensional spoiled gradient echo images. Additional images were acquired at 22 days after injection and again at 3 and 6 months after treatment. The 3-week scan was performed to assess for any early inflammatory changes potentially related to the peptide, whereas later scans were performed for surveillance of tumor status.

Injection of Radiolabeled Peptide

Patients received supersaturated potassium iodide (300 mg/kg) 1 day prior and 3 days after administration of ^{131}I-TM-601 to block uptake of ^{131}I by the thyroid gland. Patency of the venous access device was evaluated by injection of 0.5 to 1 mCi of ^{111}Indium-diethylenetriamine pentaacetate (^{111}In-DTPA) followed by gamma camera imaging of the head. Serial images were acquired every 2 minutes for 16 minutes to determine whether the ^{111}In-DTPA was leaking from the cavity site. The leakage was measured by total counts in a selected region of interest over the study period. If more than 30% of the ^{111}In-DTPA had leaked from the cavity site, administration of the study drug was aborted.

Next, patients received 25% of the total dose via injection into the venous access device, were observed for 5 minutes, and then received the remainder of the study dose. Patients were monitored during drug infusion and re-evaluated on a daily basis during the immediate (days 0 to 8) postinfusion period; patients were seen again at day 22 and then observed for up to 180 days. Biodistribution and elimination were determined by urine and blood measures of radioactivity. Twenty-four hour urine collections were performed over days 1 to 2, 2 to 3, 3 to 4, and 4 to 6 or 8, with an aliquot from each 24-hour period used to determine the average amount of excreted radioactivity during that time period. Blood samples were collected 1, 2, and 4 hours after the completion of the infusion on days 2, 3, and 4 and at the time of imaging on day 6 or 8.

Gamma Camera Imaging

Cobalt-57 transmission scan. A cobalt-57 transmission scan with and without the patient was used to obtain attenuation correction factors for total-body image quantification, as previously described.[12]

Whole-body and two-dimensional brain single photon emission computed tomography (SPECT) scans. After intracavitary injection of ^{131}I-TM-601, anterior and posterior whole-body planar images and two-dimensional SPECT scans were acquired as described.[12] A 20-mL calibrated ^{131}I source (approximately 100 μCi) was placed 10 cm from the feet of the patient within the field of view. Subsequent images were acquired on days 1, 2, and 3 and between 5 and 8 days after injection.

Study Design and Statistical Methods

The goals of this study were primarily to evaluate the safety, biodistribution, and dosimetry of a single dose of intracavitary ^{131}I-TM-601 infused into the tumor resection cavity. Because TM-601 had never been administered to humans and the toxic effects and dose-limiting toxicities (DLTs) of ^{131}I are well documented, a standard trace dose of 10 mCi ^{131}I was used for imaging as required by the US Food and Drug Administration before radioactivity dose-escalation trials. Estimates of the number of surface receptors for TM-601 on glioma cells indicated that peptide doses in the range of 0.1 to 1.0 mg were adequate to saturate all binding sites.[7] Thus, three peptide doses were chosen for dose-escalation studies, with the amount of radioactivity fixed at 10 mCi. This design was requested and approved by the US Food and Drug Administration.

Preliminary assessment of antitumor effect was a secondary end point. Six patients were enrolled onto one of three sequential dosing panels (panel 1, 0.25 mg of TM-601; panel 2, 0.50 mg of TM-601; and panel 3, 1.00 mg of TM-601), each radiolabeled with 10 mCi (± 10%) of ^{131}I. Treatment within a dosing panel would have been interrupted if two or more of the initial three patients experienced a DLT (grade 3 or higher according to the National Cancer Institute Common Toxicity Criteria version 3.0 and graded as at least probably related to treatment). Dose escalation similarly would have been interrupted if two or more DLTs occurred within a single dosing panel. If two patients at a given dose experienced a DLT, the previous dose level would have been identified as the maximum tolerated dose. Every patient was observed clinically for 180 days after treatment. All efficacy and safety analyses were performed on the intent-to-treat cohort of all patients who received a single dose of intracavitary ^{131}I-TM-601.

Radiation Dosimetry

The tissue uptake, clearance, and dosimetry of ^{131}I-TM-601 for the whole body, normal organs, and brain were determined based on five sequential, quantitative, whole-body gamma camera images as previously detailed.[13] Radiation to normal organs was calculated using the MIRDOSE III program (free software; Oak Ridge Laboratories, Oak Ridge, TN) based on the reference man.[14] Radiation dose of ^{131}I to the tissue surrounding the resection cavity was evaluated based on SPECT images. The counts in the SPECT images were converted to μCi of ^{131}I based on a calibrated imaging standard. The distribution of ^{131}I was converted to the radiation dose rate distribution using dose convolution with electron and photon dose kernel. Radiation doses to the tumor resection cavity were estimated to within 2 cm of tumor margins because most recurrences occur within this distance.[15,16]

Radioactivity concentrations in blood and urine were determined using a gamma well counter calibrated with a ^{131}I standard. For blood, total radioactivity was calculated based on the area under the radioactivity-time curve, with the typical peak (μCi/mL) at 4 hours. Cumulated activity during the 0- to 4-hour window was determined by trapezoid integration, and cumulated activity after more than 4 hours was fitted with a monoexponential curve. Marrow-to-blood ratio[17] was assumed to be 0.75 because of the small peptide size. Patient-specific marrow dose was estimated based on the electron radiation from the blood, the photon radiation from the remaining body and tumor cavity, and the patient's body weight.[18]

Histochemical Staining

A tissue sample was obtained from each patient during surgery. Each specimen was subjected to immunohistochemical staining to test for TM-601 binding. Staining followed the method of Lyons et al[19] with few modifications.

RESULTS

Nineteen patients with recurrent high-grade glioma were enrolled onto the study; 18 had glioblastoma multiforme (GBM), and one had anaplastic astrocytoma. One GBM patient was excluded after surgery

because of a diagnosis of previously undetected hepatitis C. The demographics of the patient population are listed in Table 1. All patients received at least one dose of study medication.

For unplanned reasons, two patients assigned to the 0.50-mg dose panel and one patient assigned to the 1.00-mg panel received a second dose of study medication. In one of these patients, SPECT images indicated that the first injection was accidentally delivered subcutaneously and did not enter the resection cavity. Calculated radiation doses to normal organs after this subcutaneous injection were determined to be clinically insignificant. This patient received a second injection of 10 mCi ^{131}I-TM-601 into the reservoir, confirmed by subsequent SPECT images. Two other patients received a second dose on a compassionate use basis, with approval from the US Food and Drug Administration, at 12 and 19 weeks after initial treatment. Survival from time of injection for all patients is shown in Figure 1. Two patients demonstrated a small amount of ^{111}In-DTPA leakage into the ventricles and spinal fluid pathways. Radiation dose estimates suggest that the radiation dose of ^{131}I-TM-601 to the spine was in a range thought to be clinically insignificant (2.83 Gy and 3.78 Gy). In these patients, the treating physician determined that administration of 10 mCi ^{131}I-TM-601 was still appropriate for this study.

Radiation Dosimetry

Radiation doses to normal organs were clinically insignificant (Table 2). In contrast, the mean radiation dose to within 2 cm of the cavity wall was 0.81 Gy/mCi (median, 0.49 Gy/mCi), and the dose ranged from 0.12 to 2.75 Gy/mCi (Table 2). Furthermore, the biologic half-life of ^{131}I-TM-601 in the tumor cavity margin was longer than in any other organ, indicating long-term retention of the drug in and around the injection site (Table 2). The median biologic half-life in cavity margin was 70 hours (range, 32 to 193 hours), 80 hours (range, 25 to 86 hours), and 55 hours (range, 41 to 62 hours) for patients receiving 0.25, 0.50, and 1.0 mg of peptide, respectively.

The biologic half-life, radiation dose per unit of injection dose (Gy/mCi), and radiation dose (Gy) for ^{131}I-TM-601 within the 2-cm tumor cavity wall are listed for each patient in Table 3. These data indicate a slightly longer half-life and higher radiation dose for patients receiving 0.50 mg of peptide compared with the other groups, although this difference did not reach statistical significance. ^{131}I-TM-601 localized to and remained primarily concentrated in and around the patients' surgical cavity for all 5 days that the patients were imaged (a typical image is shown in Fig 2).

Table 1. Patient Characteristics

Characteristic	Overall	Panel 1	Panel 2	Panel 3
Sex, No. of patients				
Female	7	2	2	3
Male	8	2	3	3
Age, years				
Mean	47.2	44.5	46.3	50.7
Standard deviation	10.6	14.7	9.6	10.7
Race/ethnicity, No. of patients				
White	13	3	5	5
Hispanic	1	0	0	1
Black	1	1	0	0
Asian	0	0	0	0
Karnofsky performance status, %				
Mean	82.8	83.3	81.7	83.3
Standard deviation	11.3	15.1	11.7	8.2
Histology, No. of patients				
Glioblastoma	12	3	4	5
Anaplastic astrocytoma	3	1	1	1
Tumor location, No. of patients				
Frontal lobe	10	6	0	4
Temporal lobe	5	0	4	1
Parietal lobe	3	0	2	1
Prior therapy, No. of patients				
Radiation	15	5	6	4
Chemotherapy	15	5	6	4

Patient Follow-Up, Toxicity, and Response to Therapy

Eleven patients completed the 180-day observation period. There were no DLTs related to treatment during the initial 22-day observation period and no clinically significant acute adverse events during infusion of ^{131}I-TM-601 at any dose level. The majority of events reported were mild to moderate in nature. There were no grade 3 or 4 toxicities related to the study drug or method of administration in the immediate and/or long-term follow-up period. There were 88 grade 1 and 90 grade 2 toxicities. There were no patient complaints related to the study drug or method of administration.

Four patients had serious adverse events possibly or probably related to study medication reported within 22 days of administration (Table 4). Additional serious adverse events reported beyond the initial 22-day observation period included one patient with generalized seizure and increased confusion; one patient with pneumonia; one patient with somnolence, ventricular dilation, and cerebral hematoma; and one patient with headache, dysarthria, and instability. The administration of a second dose of study medication was not associated with any serious adverse events, although these events were not formally included in the toxicity evaluations because of the long time interval (12 and 19 weeks) between drug administrations.

Over the course of the 180-day observation period, there were seven deaths. Two patients in panel 2 with GBM have survived more than 30 months. Median survival time was 25.7 weeks for patients in panel 1 (0.25-mg dose), 77.6 weeks for patients in panel 2 (0.50-mg dose), 23.6 weeks for patients in panel 3 (1.00-mg dose), and 27.0 weeks for patients in all three dosing groups (Table 5). Histochemistry of the tumor tissue from all patients stained intensely positive for TM-601, as represented in Figures 3A to 3C.

Radiographic Changes

Tumor volume measurements were available for 16 patients at baseline (within 48 hours of surgery), 16 patients at 22 days after treatment, 16 patients at 90 days after treatment, and five patients at 180 days after treatment. All but one patient had evidence of residual enhancing disease on baseline scans. The mean baseline residual T1 enhancing tumor volume was 28 ± 28 mL (range, 0 to 72.15 mL). On day 22 after treatment, this volume had increased to a mean of 31.8 ± 32.7 mL (range, 1.8 to 114.2 mL). The tumor volumes decreased by 10.8% in one patient and 76.7% in another patient, were unchanged in nine patients, and increased in three patients (four patients were not assessable). This translated into a radiographic interpretation of stable disease in 12 patients and progressive disease in four patients (two patients were not assessable at this time point; Table 4). For 16 patients with radiographic follow-up available at 90 days, a stable response was observed in seven patients, and progressive disease

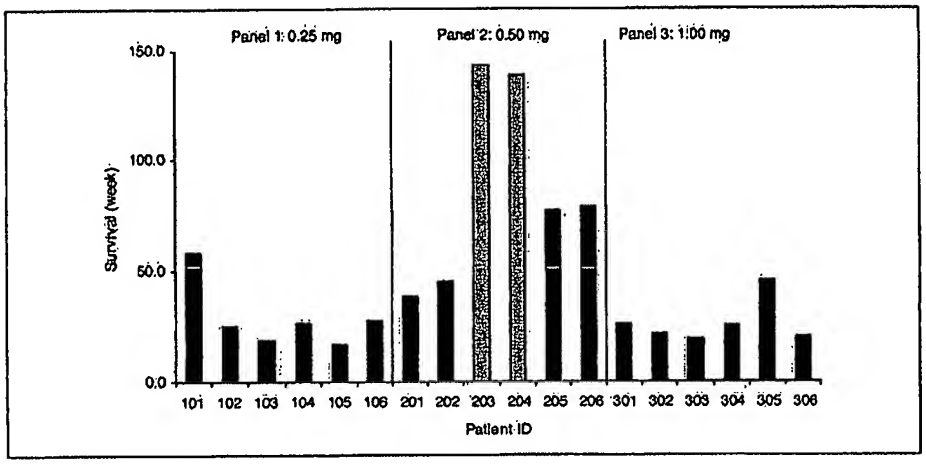

Fig 1. Individual patient survival. Length of survival for each patient (as of November 2005) after treatment with 10 mCi iodine-131-TM-601 measured in weeks.

was observed in nine patients (two patients were not assessable at this time point). Long-term follow-up was available for six patients, with one patient showing a partial response (defined as at least a 50% decrease under baseline with no new lesions), four with stable disease, and one with progressive disease. Two patients (one with stable disease and one with partial response) went on to achieve a complete radiographic response (defined as complete absence of demonstrable contrast enhancement on T1-weighted MRI) without evidence of disease for 32 and 30 months. The patients (patients 203 and 204) were females and were ages 40 and 42 years. Both patients had parietal lobe GBM (one left hemisphere and one right hemisphere), a KPS of 90% after resection, and minimal residual enhancement on postoperative MRIs. Neither patient received a second dose of ^{131}I-TM-601. An example of stable disease (patient 204) is demonstrated in Figures 3D and 3E.

DISCUSSION

In this first human trial, treatment of patients with recurrent high-grade glioma with a single intracavitary dose of ^{131}I-TM-601 was well tolerated to the dose of 1.0 mg TM-601 radiolabeled with 10 mCi of ^{131}I. Few adverse effects occurred during the initial 22-day observation period, which suggests the dosing level of peptide used in this study is safe and well tolerated. It is unlikely that the doses of ^{131}I contributed to the adverse events because the doses were far below expected toxicity ranges.[20] The adverse events that did occur were considered unremarkable in this patient population. Three patients received two doses without any significant adverse events, preliminarily demonstrating that repeated administration of ^{131}I-TM-601 may be safe.

Biodistribution data of ^{131}I-TM-601 indicated that this radiopeptide rapidly penetrated through the cavity wall with, on average, 79% of the radioactivity leaving the region of cavity within 24 hours after administration. The majority of the remaining radioactivity stayed tightly localized to the tumor cavity and surrounding regions, suggesting discrete binding to the tumor. The amount of uptake and radiation

Table 2. Organ Dose and Half-Life

Organ	Dose (Gy/mCi) Mean	Dose (Gy/mCi) Range	Biologic Half-Life (hours) Mean	Biologic Half-Life (hours) Range
Marrow, blood	0.001	0.002-0.012	7	3-18
Stomach	0.007	0.002-0.013	25	17-40
Kidney	0.009	0.002-0.018	26	10-39
Thyroid	0.053	0.008-0.229	NA	—
Normal brain	0.001	0.000-0.024	2	0-189
Marrow, blood half-life	0.003	0.001-0.004	29	9-41
Cavity wall	0.32	0.01-0.96	NA	—
Tumor cavity wall	0.81	0.12-2.75	69	25-193

Abbreviation: NA, not available.

Table 3. Individual Patient Dosimetry and Half-Life of ^{131}I-TM-601 at Tumor Cavity Wall

Dose and Patient No.	Biologic Half-Life (hours)	Average Biologic Half-Life (hours) Mean	Average Biologic Half-Life (hours) Range	Dose per Unit InjectionDose (Gy/mCi)	Average Dose (Gy) Mean	Average Dose (Gy) Range
0.25 mg		67.6	39.2-108.4		6.88	1.26-25.30
101	32.1			0.122		
102	63.5			0.043		
103	199.1			0.734		
104	40.2			0.391		
105	47.16			2.08		
106	100.0			2.748		
0.50 mg		79.8	25.1-86.4		7.29	2.80-12.96
201	86.4			0.906		
202	78.9			0.376		
203	55.2			1.168		
204	25.1			0.851		
205	80.8			0.667		
206	85.6			0.253		
1.00 mg		55.7	40.5-61.7		4.26	3.45-8.48
301	52.3			0.440		
302	57.5			1.899		
303	46.8			0.42		
304	40.5			0.331		
305	60.3			3.048		
306	61.7			0.529		

Abbreviation: ^{131}I, iodine-131.

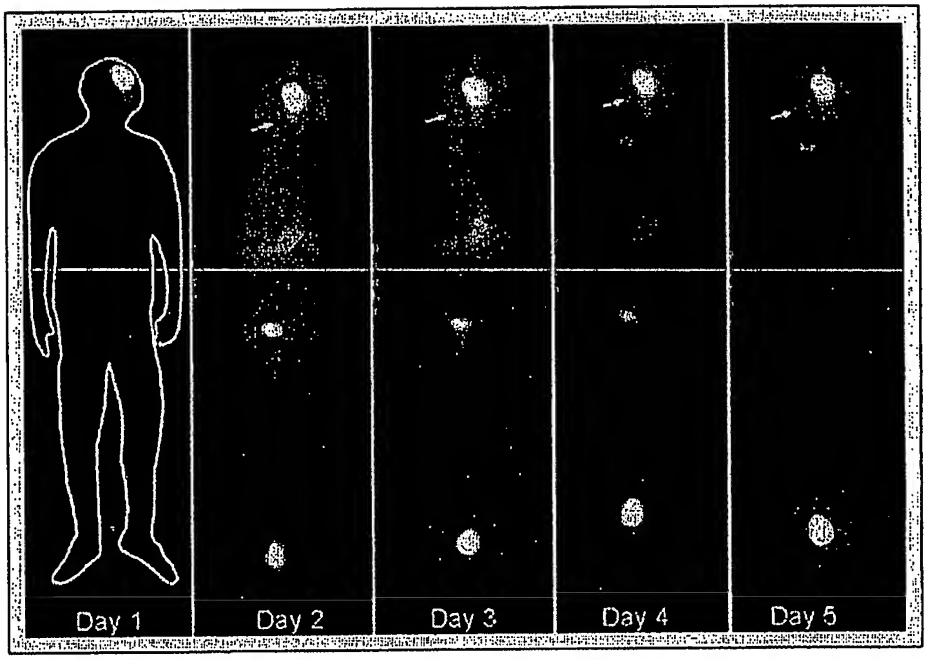

Fig 2. Gamma camera scans. Anterior gamma camera scans for patient 104. Intense localization is seen in the surgical cavity as late as 5 days after infusion. This result was confirmed on brain single photon emission computed tomography scans and was observed uniformly in all patients. Note the location of hot spots outside the surgical cavity (arrows).

doses in the stomach, kidneys, spleen, and bladder were much lower compared with those reported in the literature for other modalities.[21,22] There was no observable uptake of ^{131}I-TM-601 in the small or large intestine at any time in any patient, suggesting that the excretion route of ^{131}I-TM-601 is mainly through the urinary tract. Uptake of ^{131}I-TM-601 in the thyroid was greater than in other solid organs but still far below toxic levels, which are reported to be in the range of 100 Gy to cause hypothyroidism in greater than 50% of patients.[23,24] Immunohistochemical studies in normal human tissues have failed to demonstrate TM-601 binding in normal thyroid gland,[7] which is consistent with our observation.

A detailed analysis of the imaging of this drug in the brain based on a subset of nine patients has been published,[12] indicating that ^{131}I-TM-601 diffuses into the brain at distances far greater than observed for antibodies and other large molecules.[25] These observations

Table 4. Adverse Events

Event	Severe Adverse Event Grade	No. of Occurrences
All events		
Eye pain		
Nausea		
Asthenia		
Neck pain		
Aphasia		
Hemiparesis		3
Paresthesia		
Agitation		
Anxiety		
Depression		
Meningoencephalitis		
Infection		
Altered liver function		
Headache		
Cerebral edema		
Events likely related to study drug		
Infection	1	1
Paresis	1	3
Cerebral edema	1	1

*One patient each had candidal infection and herpes zoster.

Table 5. Tumor Response and Survival

Patient No.	Tumor Response by MRI			Survival Time (weeks)
	Day 22	Day 90	Day 180	
101	PD	PD	NA	8.1
102	SD	PD	NA	25.0
103	PD	NA	NA	8.6
104	SD	SD	SD	26.4
105	NA	PD	NA	7.0
106	SD	SD	NA	27.2
201	SD	SD	PD	38.1
202	NA	PD	NA	44.9
203	SD	SD	SD	38.3
204	SD	SD	PR	133.7
205	SD	SD	SD	76.7
206	SD	PD	NA	78.6
301	PD	PD	NA	8.6
302	SD	PD	NA	21.9
303	SD	SD	SD	109.3
304	SD	PD	NA	25.4
305	SD	PD	NA	15.0
306	PD	NA	NA	20.1

Abbreviations: MRI, magnetic resonance imaging; PD, progressive disease; NA, not available; SD, stable disease; PR, partial response.
*Received second therapeutic dose of iodine-131-TM-601 on a compassionate use basis.

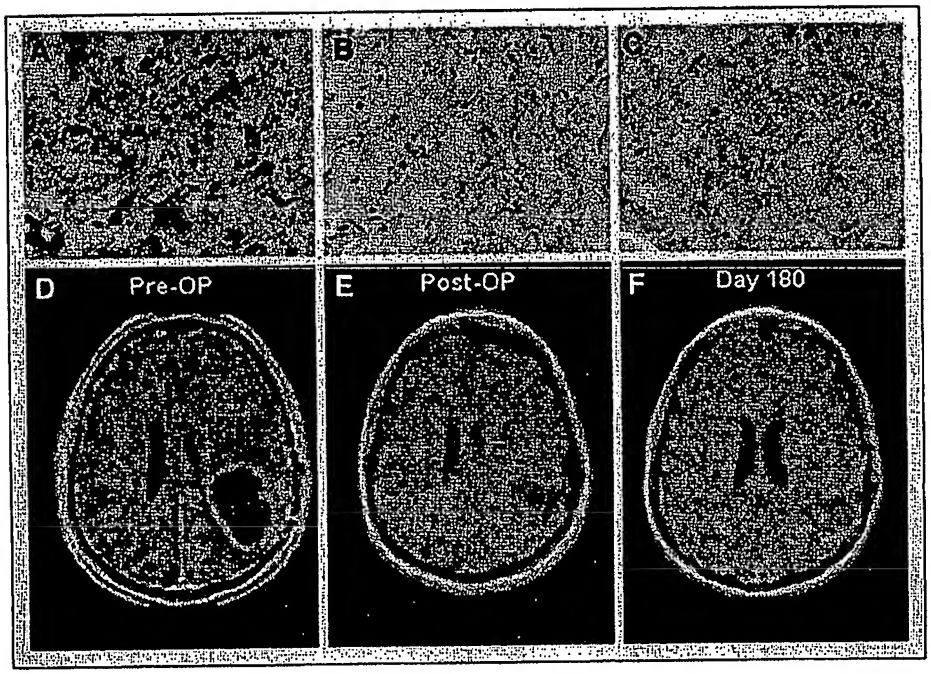

Fig 3. Histology and radiographic response. Representative histology of a sample from a patient with glioblastoma multiforme (patient 0204) is shown. Frozen sections were fixed and stained with biotinylated peptides. Streptavidin-horseradish peroxidase and diaminobenzidine were used for detection as indicated by the intense brown staining with biotinylated TM-601 in panel A. (A) Biotinylated TM-601 (10 μmol/L). (B) No peptide-negative control. (C) Hematoxylin and eosin stain. Radiographic response of this patient is shown in panels D, E, and F, indicating stable disease at 180 days after treatment.

suggest that TM-601 may be a useful means to deliver focused radiotherapy to patients with glioma. Limited by current state of the art imaging technologies and because of the nonuniform microscopic distribution of ^{131}I-TM-601 and residual tumor cells, the current macroscopic radiation dose calculations based on imaging may not accurately represent the actual radiation dose delivered to tumor cells.[26]

In two GBM patients receiving 0.5 mg TM-601 plus 10 mCi ^{131}I ($\pm$ 10%), a complete radiographic response was observed. These two patients are still alive 37 and 39 months after surgery (as of March 2006) even with this low dose of peptide and expected subtherapeutic level of radiation. Of note, these patients were slightly younger than the average patient in the study (ages 40 and 42 years) but were otherwise quite representative of the remainder of the study cohort. Thus, although we acknowledge that confounding factors, such as patient age, tumor size, extent of resection, and KPS, may have contributed to this result,[27] the responses certainly suggest that further investigation of this minimally toxic agent is warranted.

Intracavitary-administered ^{131}I-TM-601 is simple to deliver, well tolerated, remains highly localized to the treatment site, and preliminarily seems safe for repeated injections. Recently, a phase II trial has been initiated using escalating peptide and radiation doses with multiple injections for patients with high-grade glioma.

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Authors' Disclosures of Potential Conflicts of Interest

Although all authors completed the disclosure declaration, the following authors or their immediate family members indicated a financial interest. No conflict exists for drugs or devices used in a study if they are not being evaluated as part of the investigation. For a detailed description of the disclosure categories, or for more information about ASCO's conflict of interest policy, please refer to the Author Disclosure Declaration and the Disclosures of Potential Conflicts of Interest section in Information for Contributors.

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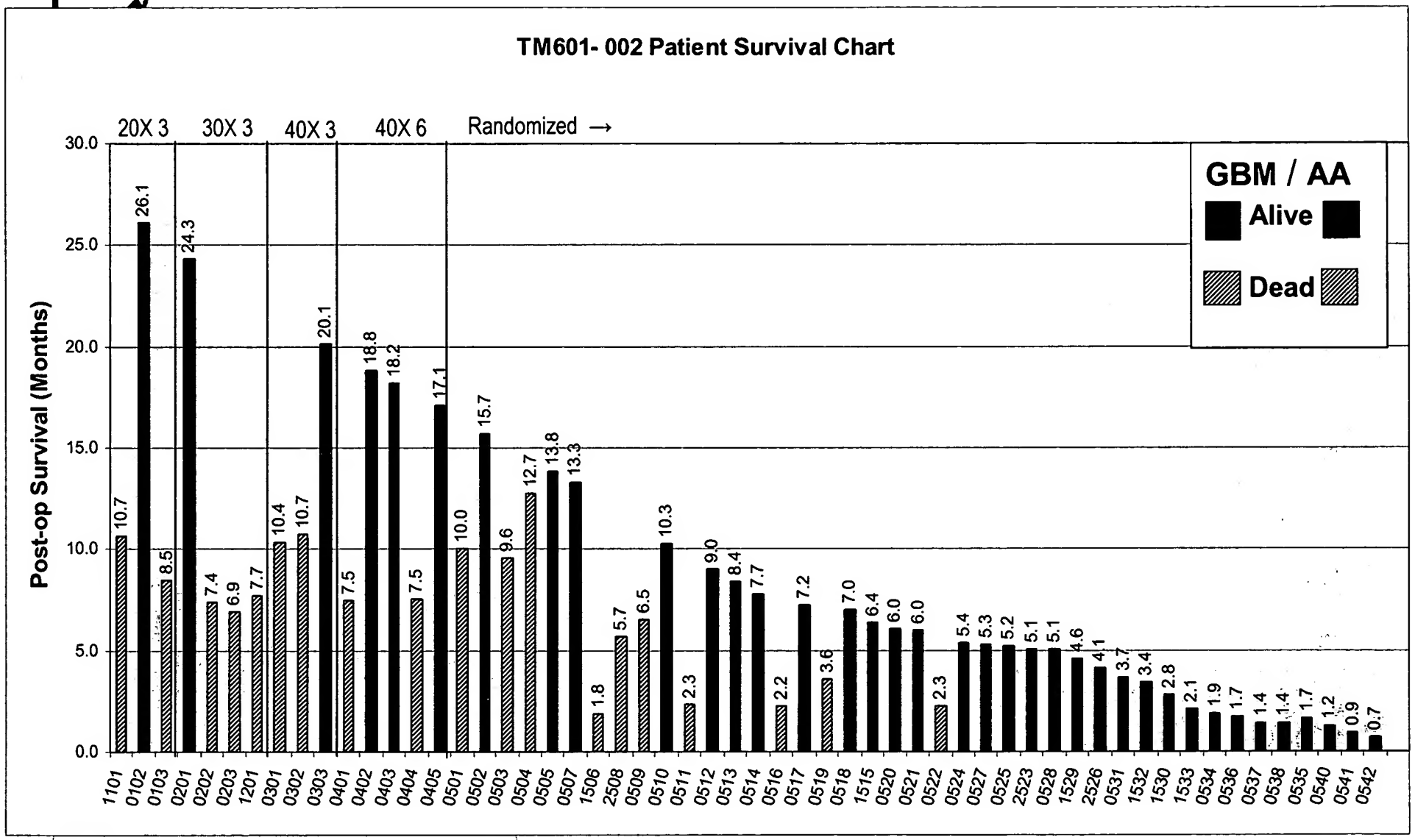
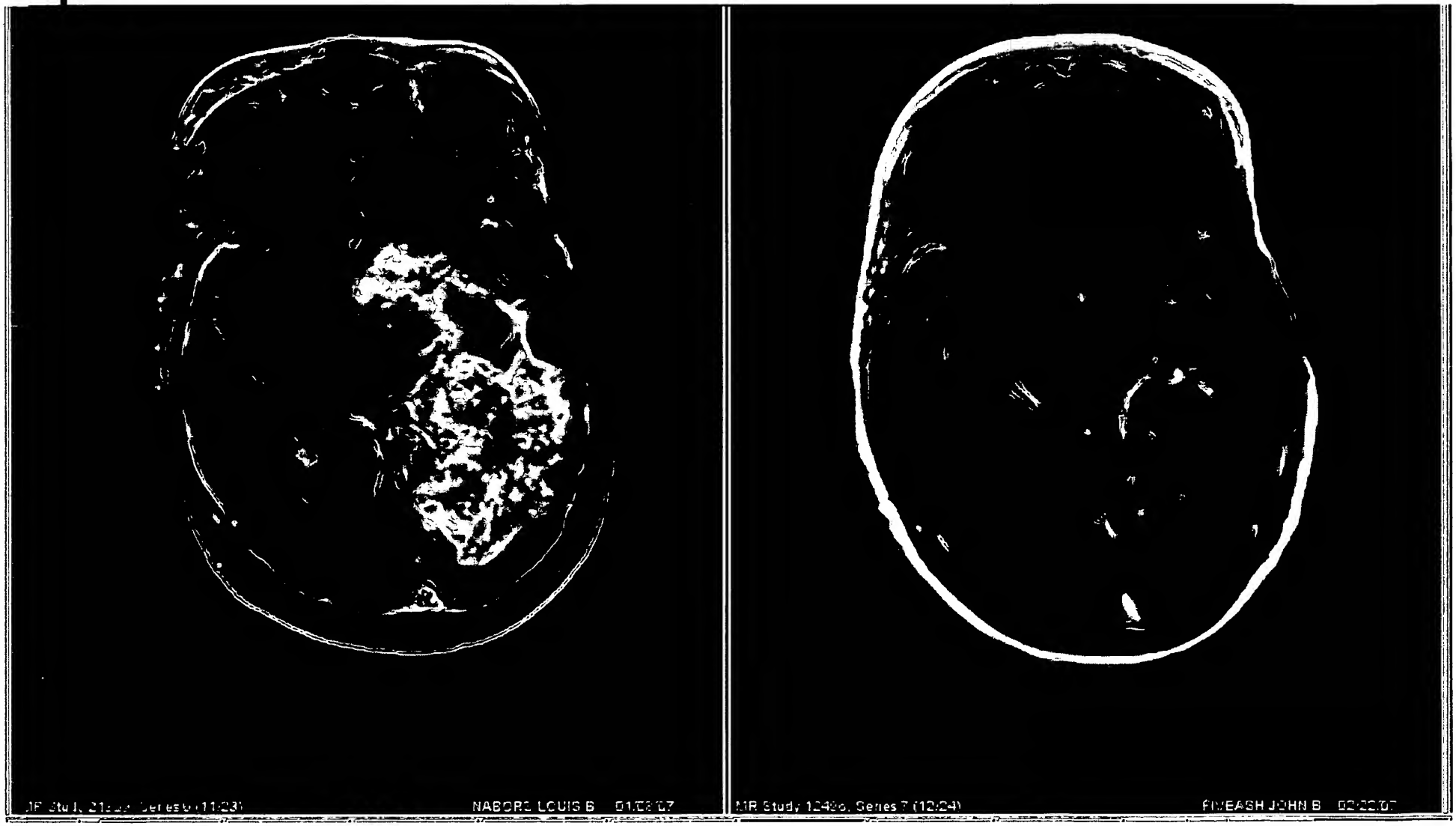


Status as of April 4, 2007

6 month survival = 31/36 (86%)
9 month survival = 17/27 (63%)
12 month survival = 10/22 (45%)

Exhibit C

IV Injection in Malignant Glioma



Pt 01-007: Pretreatment MRI (1/8/2007)

- Confidential -

3 weeks following 30 mCi dose (2/22/2007)

Exhibit D